PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/165 — A1

(11) International Publication Number: WO 94/22436

(43) International Publication Date: 13 October 1994 (13.10.94)

(21) International Application Number: PCT/US94/03355

(22) International Filing Date: 31 March 1994 (31.03.94)

(30) Priority Data:
08/040,870 31 March 1993 (31.03.93) US

(71)(72) Applicant and Inventor: COOPER, George, IV [US/US]; 23 Savage Street, Charleston, SC 29401 (US).

(74) Agents: KILLOUGH, Billy, C. et al.; P.O. Drawer H, Charleston, SC 29402 (US).

(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: A METHOD FOR TREATING ABNORMAL CARDIAC CONTRACTION

(57) Abstract

Cardiac contractile dysfunction is specific to stress loading. Microtubules which are adjacent to the myofilaments polymerize in response to stress loading, and account for contractile dysfunction in pressure overloading cardiac hypertrophy. Microtubular depolymerization reverses the contractile abnormality of the pressure hypertrophied cardiocyte.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/165 — A1

(11) International Publication Number: WO 94/22436

(43) International Publication Date: 13 October 1994 (13.10.94)

(21) International Application Number: PCT/US94/03355

(22) International Filing Date: 31 March 1994 (31.03.94)

(30) Priority Data:
08/040,870 31 March 1993 (31.03.93) US

(71)(72) Applicant and Inventor: COOPER, George, IV [US/US]; 23 Savage Street, Charleston, SC 29401 (US).

(74) Agents: KILLOUGH, Billy, C. et al.; P.O. Drawer H, Charleston, SC 29402 (US).

(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: A METHOD FOR TREATING ABNORMAL CARDIAC CONTRACTION

(57) Abstract

Cardiac contractile dysfunction is specific to stress loading. Microtubules which are adjacent to the myofilaments polymerize in response to stress loading, and account for contractile dysfunction in pressure overloading cardiac hypertrophy. Microtubular depolymerization reverses the contractile abnormality of the pressure hypertrophied cardiocyte.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

A METHOD FOR TREATING ABNORMAL CARDIAC CONTRACTION

BACKGROUND OF THE INVENTION

Cardiac hypertrophy is the generic response to a wide spectrum of physiological and pathological deviations from normal homeostasis that have as their common theme increased hemodynamic loading of the heart. This compensatory growth process proceeds until the load stimulus is abated via a re-normalization of stress per unit of myocardial mass. Cardiac hypertrophy fails to be functionally compensatory, however, either when the load increase exceeds the inherent growth capacity of the terminally differentiated cardiac muscle cell, or cardiocyte, to re-normalize stress or when the intrinsic contractile performance per unit mass of hypertrophied myocardium is less than that of normal myocardium. Thus cardiac compensation for an increased load may be imperfect because of either quantitative and qualitative defects of hypertrophied myocardium.

Hemodynamic overloads causing cardiac hypertrophy consist of either volume overloading, wherein an increased blood volume is pumped during each cardiac cycle against a normal impedance, or pressure overloading, wherein a normal blood volume is pumped during each cardiac cycle against an increased impedance. The inventor has observed that for an equivalent degree and duration of hypertrophy, volume overloading results in entirely normal cardiac contraction and energetics, while pressure overloading results in distinctly abnormal cardiac contraction and energies, a result consonant with clinical experience with the intact right ventricle. On the level of isolated right ventricular cardiocytes, the same model of right ventricular pressure overload that the contractile defect seen in isolated tissue is duplicated when characterized as sarcomere shortening in the muscle cell. Thus it is the nature of the inducing stress rather than hypertrophy itself that causes the qualitative defects of myocardium hypertrophying in response to a pressure overload, and the contractile defect, at least, resides in the cardiocyte.

Cardiocyte structure, composition, and function each respond dynamically to the full potential spectrum of imposed loads, with deviations either below or above normal loading

causing rapid but reversible changes in each of these properties. The cardiocyte itself is competent to respond directly to load in terms of RNA and protein synthesis rates. However, there are neither qualitative nor quantitative differences between hypertrophied cardiocytes of pressure versus volume overloaded right ventricles when defined in terms of standard ultrastructure, yet the contractile defect is expressed quite clearly in the pressure overloaded cell.

The microtubular component of the cytoskeleton is an intracellular structure, which, in excess, is responsible for the contractile abnormalities of cardiocytes hypertrophying in response to a pressure overload. Therefore, microtubules are increased in the pressure hypertrophied cardiocyte, and any contractile abnormality which this might cause is fully reversed when the microtubules are depolymerized by either chemical or physical agents. Further, at the level of the isolated cell, it is appropriate to extend such a treatment stratagem to the pressure hypertrophied heart exhibiting contractile dysfunction *in vivo*, especially in the case of clinical disease states.

There is a persistent increase in polymerized tubulin in cardiocytes that hypertrophy in response to a stress but not a strain overload. The contractile defect exhibited by these cells is fully reversed when the microtubules are depolymerized by either chemical (e.g. colchicine) or physical (e.g. hypothermia) agents. Thus, this fully reversible cytoskeletal alteration accounts for the entirety of the contractile abnormality observed on the cellular level in the pressure overloaded right ventricle. This is equally true for cardiocytes isolated from the pressure hypertrophied, dysfunctional left ventricle.

SUMMARY OF THE INVENTION

Cellular and ventricular contractile function are normal in right ventricular (RV) volume overload (VO) and abnormal in RV pressure overload (PO) that is, contractile dysfunction is specific to stress loading. Microtubules (MTs), which are adjacent to the myofilaments, polymerize in response to stress loading. Cardiac muscle cells,or cardiocytes, are

enzymatically isolated from each ventricle with RVPO (pulmonary artery band) or RVVO (atrial septal defect). MT depolymerization by colchicine (10^{-6}M for 1 hr) normalizes contractility in RVPO cells but had little effect on either hypertrophied RVVO or normal LV cells. Cooling cells to 0°C for 1 hr, which also depolymerizes MTs without affecting intermediate filaments, has the same normalizing effect on contractile dysfunction as did colchicine. Cytochalasin D, which depolymerizes microfilaments, is without effect on contractile function. In contrast, taxol (10^{-5}M for 3 hr) or 50% D_2O, which stabilize or polymerize MTs, decrease sarcomere motion in normal cells to an extent comparable to that seen in untreated RVPO cells. Thus microtubules, a load responsive intracellular structure, account for the contractile dysfunction of PO cardiac hypertrophy. Further, microtubular depolymerization completely reverses the contractile abnormality of the pressure hypertrophied cardiocyte.

DESCRIPTION OF THE PREFERRED EMBODIMENT

In the face of a hemodynamic overload, the need for the heart to provide adequate systemic blood flow is accomplished via an increase in the mass of the cardiac pump. This process, cardiac hypertrophy, is thus the basic compensatory mechanism for a variety of human disease states in which the active stress per unit mass of the myocardium of an entire cardiac ventricle is increased (hypertension or valvular stenosis) or the active stress per unit mass of remaining myocardium is increased after part of the myocardium is lost (myocardial infarction). Cardiac hypertrophy is fully compensatory, when contractile function of the enlarged myocardium remains normal. However, this compensation fails when the contractile function per unit mass of enlarged myocardium becomes abnormal. This decompensation of contractile function is the major underlying etiology of the congestive heart failure state, an entity which in patients is currently a leading cause of death and debility. In pressure overload cardiac hypertrophy there is a contractile defect at the levels of isolated cardiac tissue and muscle cells from that tissue. Thus the contractile defect observed in the intact

organism can be attributed to a cellular defect. Identification of that cellular defect provides the opportunity for the development of a specific therapy for the contractile dysfunction seen in some forms of cardiac hypertrophy. An excess of microtubules is a cellular defect in pressure hypertrophied myocardium. When the microtubules are removed via depolymerization, the contractile defect is, *pari passu*, entirely removed. What is true for the pressure hypertrophied right ventricle is equally true for the pressure hypertrophied left ventricle. That is, when left ventricle is pressure overloaded, left ventricular hypertrophy ensues, and this is accompanied by contractile dysfunction as the degree of left ventricular hypertrophy increases, a situation quite similar to that seen in patients with pressure overload left ventricular hypertrophy. When the cardiac muscle cells are isolated from left ventricles, decreased cellular contractile function is observed. This is associated with increased microtubules. Microtubular depolymerization returns the cellular contractile function to normal.

In practice, an agent which will depolymerize microtubules is introduced into hypertrophied cardiocytes. Preferred depolymerizing agents are those which will bind to the tubular monermers and prevent the formation of microtubule polymers. The depolymerizing agent could be colchicine.

Introduction of the agent may be localized by known means of introducing chemicals or drugs into cardiocytes. Introduction of the agent may be systemic by known means such as intravenous, intramuscular or oral means.

It is acknowledged that an agent such as colchicine, which will depolymerize microtubules, has high systemic toxicity and will not permit *in vivo* use when introduced systemically.

Depolymerization of microtubules may be achieved by cooling cardiocytes by exposure to 0°C for 1 hour. The cold depolymerized microtubules will achieve normal contractile function, as seen when microtubules are depolymerized chemically, such as by colchicine. When the cardiocytes are studied at 37°C (T0), then studied again at 37°C after an intervening hour at 0°C (T1), and then studied again after a further hour of 37°C (T2),

the initially abnormal contractile function of right ventricular cardiocytes are normalized at T1 and remain the same as that for left ventricular cardiocytes at T2; both right ventricular and left ventricular cardiocytes show a modest decrement and contractile function during microtubular repolymerization between T1 and T2 similar in degree to the increment and contractile function seen for normal cardiocytes during microtubular depolymerization. Of particular interest is the fact that when cardiocyte microtubules which polymerized under a stress load *in vivo* polymerized under zero load *in vitro* the initial contractile abnormality was not recapitulated.

WHAT IS CLAIMED IS:

1. A method for treating abnormal cardiac contraction, comprising the steps of introducing a depolymerizing agent into microtubules of hypertrophied cardiocytes wherein the microtubules are depolymerized so as to restore normal cardiac contractile function.

2. A method for treating abnormal cardiac contraction as described in claim 1, wherein said depolymerizing agent binds to tubular monermers to prevent said microtubules from forming polymers.

3. A method for treating abnormal cardiac contraction as described in claim 2, wherein said depolymerizing agent is colchicine.

4. A method for treating abnormal cardiac contraction as described in claim 1, wherein said depolymerizing agent is introduced locally into said cardiocytes.

5. A method for treating abnormal cardiac contraction as described in claim 2, wherein said depolymerizing agent is introduced locally into said cardiocytes.

6. A method for treating abnormal cardiac contraction as described in claim 1 wherein the depolymerizing agent is introduced systemically.

7. A method for treating abnormal cardiac contraction as described in claim 2 wherein the depolymerizing agent is introduced systemically.

8. A method for treating abnormal cardiac contraction comprising the steps of first reducing the temperature of microtubules of hypertrophied cardiocytes from normal body temperature to a temperature which will cause said microtubes to

depolymerize and subsequently restoring the cardiocytes to normal body temperature.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03355

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :A61K 31/165
US CL :514/629
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/629

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biological Abstracts, Volume 93, No. 10, issued 15 May 1992, Lampidis et al, "Cardiostimulatory and antiarrhythmic activity of tubulin-binding agents", see page 859, column 2, abstract no. 115017, Proc. Natl. Acad. Sci. USA, 89(4), 1256-1260.	1-8
Y	International Review of Cytology, Volume 113, issued 1988, Rappaport et al, "Microtubules in Cardiac Myocytes", pages 101-143, see particularly pages 124 and 126.	1-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
04 MAY 1994

Date of mailing of the international search report
23 JUN 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
WILLIAM JARVIS
Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03355

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN(CAS, BIOSIS, MEDLINE)
search terms: colchicine, microtubules, cardiac, heart, polymerize, depolymerize, hypertrophy, contractile, temperature, hypothermic